TEST SPECIFIC CHECKLIST

May 1999

Test of Sexual Reproduction Using the Red Macroalga *Champia parvula*

Parameter	Specification	Met Specifics? Y	N	NA
Sample Preparation				
T° Measurement	T° to be measured in sample on arrival at lab.	...	...	...
Filtering	If indigenous organisms, filter through a sieve (60 µm) **(Must)**.	...	...	...
T° Adjustment	Sample adjusted to 23 ± 1°C prior to test initiation (approximately 1h).	...	...	...
Salinity Adjustment	Salinity of each sample measured before starting the test.	...	...	...
	Sample adjusted to 28 - 32 g/kg using hypersaline brine (HSB) (as per EC guidance on salinity adjustment) **(Must)**.	...	...	...
pH Measurement	pH measured in each sample before test solutions are made.	...	...	...
pH Adjustment	None; a second (pH adjusted) test might be run if pH is outside 6.0 to 9.0.	...	...	...
Pre-aeration	D.O. measured in each sample; no pre-aeration unless D.O. < 4 mg/L or > 100%, then aerate all test solutions **(Must)** for a few minutes at a rate not exceeding 100 bubbles/min, until the D.O. is ≥ 4mg/L.	...	...	...
Test Conditions				
Facility	Isolated from general laboratory disturbances.	...	...	...
	Instruments available to measure basic water quality variables (T°, D.O., pH, salinity) and lab prepared for other analyses.	...	...	...
Test Type	Static.	...	...	...
Duration	2-day effluent exposure followed by 5 to 7-day recovery period in recovery medium for cystocarp development **(Must)**.	...	...	...
Temperature	23 ± 1 °C **(Must)**.	...	...	...
Light Quality	Cool-white fluorescent.	...	...	...
Light Intensity	75 µE/m^2/s.	...	...	...
Photoperiod	16 ± 1h light; 8 ± 1h dark.	...	...	...
Salinity	28 - 32 g/kg; preferably 30 g/kg; each test solution within 1 g/kg of the control; adjust using HSB (with a salinity of 90 ± 1g/kg) or deionized water.	...	...	...
	Nominal test conc. adjusted and reported in consideration of any salinity adjustments and nutrients additions **(Must)**.	...	...	...
D.O. Range	D.O. in test solutions not be permitted to fall below 4 mg/L **(Must)**.	...	...	...
Aeration	None during the exposure period, unless D.O. < 4 mg/L, then aerate all chambers at a rate not exceeding 100 bubbles/min; recommend aeration during the 5 - 7 day recovery period if shaker not used.	...	...	...
Shaker	Gently hand swirl the chambers 2X a day or shake continuously at 100 rpm on a rotary shaker during the exposure period.	...	...	...
Vessel Size & Type	200 mL polystyrene cups or 250 mL Erlenmeyer flasks; covered.	...	...	...
Test Volume	≥ 100 mL **(Must)**.	...	...	...
Renewal of Solution	None during the 2-day exposure period; at 48 h, female branches are removed from test medium chambers and place into recovery medium bottles.	...	...	...
Dilution/Control Water	Filtered (60 µm) uncontaminated lab seawater, reconstituted seawater, or filtered (60 µm) upstream receiving water.	...	...	...
	Salinity: 28 - 32 g/kg **(Must)**; recommend 30 g/kg; salinity adjusted using aged HSB with a salinity of 90 ± 1g/kg or deionized water, distilled water or uncontaminated freshwater.	...	...	...
	Any HSB used, be from the same source as that used to adjust the salinity of the sample or test solutions **(Must)**.	...	...	...
	Adjusted to 23 ± 1°C before use.	...	...	...
	If the test organisms have been cultured in water which is different from the test control/dilution water, a second set of controls, using culture water, is to be included in the test.	...	...	...
	If any HSB is added to sample or test solutions to adjust salinity, the toxicity test include a set of controls prepared using only this HSB and deionized water, adjusted to the test salinity 30 ± 2 g/kg **(Must)**.	...	...	...
	If uncontaminated receiving water used as control/dilution water, an additional lab seawater control is to be run **(Must)**.	...	...	...
	Any test using dilution water (eg: natural seawater) which differs from this HSB control include a separate set of controls prepared using this same dilution water **(Must)**.	...	...	...

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Parameter	Specification	Met Specifics? Y	N	NA
Test Medium	Test medium has the same type of nutrients as culture medium (but different concentrations) except both EDTA and trace metals are omitted (composition also described at Table 1 of the USEPA document) **(Must)**.	...	...	...
Recovery Medium	Could be test medium or culture medium; Since culture medium is a more complete nutrient medium, it may result in faster growth and faster cystocarp development during the recovery period.	...	...	...
Recovery Period	If there is uncertainty about the identification of an immature cystocarp (or a young branch), it is necessary only to aerate the plants a little longer in the recovery bottles; No new cystocarps will form since the males have been removed, and the plants will only get larger.	...	...	...
Vessel Identification	Test chambers labelled with test conc. and replicate number.	...	...	...
# Test Conc.	≥5 plus control to calculate ICp **(Must)**; dilution factor ≥ 0.5.	...	...	...
	1 plus control for single conc. test.	...	...	...
# Replicates/Conc.	≥3 replicates per conc. and control(s) **(Must)**; 4 replicates recommended.	...	...	...
	Test start with equal number of replicates for each test conc. and controls.	...	...	...
# Organisms/Vessel	5 female branches and 2 male branches per test chamber **(Must)**.	...	...	...
	Organisms that are dropped or touch dry surfaces or are injured during handling are to be discarded **(Must)**.	...	...	...
	Prepare plant cuttings using fine-point forceps with the plants in a little seawater in a petri dish; Female cuttings are severed 7 to 10 mm from the ends of the branch; Male cuttings are severed 2 to 3 cm from the ends of the branch; Be consistent in the number of branch tips on each cutting.	...	...	...
	Prepare the female cuttings first, to minimize the chances of contaminating them with water containing spermatia from the male stock cultures.	...	...	...
	The toxicant is to be present before the male plants are added **(Must)**.	...	...	...
Vessel Randomization	Randomize the position of test chambers at the beginning of the test **(Must)**.	...	...	...
Vessel Cleaning	All non-disposable test vessels and equipment to be thoroughly cleaned and rinsed in accordance with section 5.3 **(Must)**.	...	...	...
Recovery period	At 48 h, gently remove with forceps, the female branches from test chambers, rinse them in clean seawater, and place into recovery bottles containing control medium for 5 to 7 days under same lighting and T° conditions than during the test and with aeration or shaker (100 rpm).	...	...	...
Endpoints	Sexual Reproduction based on the reduction in cystocarp production (mean number of cystocarps) compared to controls; if multi conc. test, ICp (with its 95% confidence limits) is to be calculated **(Must)**.	...	...	...
Observations & Measurements				
D.O., pH, T°, Salinity	Measured at 0h and 48h of the exposure period and at the beginning and end of the recovery period in representative solutions (high, medium, low, and controls) **(Must)**.	...	...	...
Mortality	# of dead plants (female) in each test vessel at the end of the recovery period **(Must)**.	...	...	...
Cystocarps	# of cystocarps per female cutting at the end of the recovery period in each test vessel **(Must)**.	...	...	...
Test Organisms				
Species	*Champia parvula* **(Must)**.	...	...	...
Source	From in-house cultures or commercial suppliers	...	...	...
	Be identified to species **(Must)**; confirmed by a taxonomic expert.	...	...	...
Age	Sexually mature male and female branches **(Must)**.	...	...	...
	In a given test, all organisms are to be approximately the same age and be taken from the same source.	...	...	...
Health Criteria	Female plants should have trichogynes.	...	...	...
	Male plants should have sori with spermatia.	...	...	...

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Parameter	Specification	Met Specifics? Y	N	NA
Health Criteria (con't).	A group of organisms not to be used for a test if they appear to be unhealthy, discoloured, or otherwise stressed preceding the test **(Must)**.	...	...	...
	Upon failure of these criteria, the entire group is to be discarded and a new group obtained **(Must)**.	...	...	...
Culture/Holding Conditions				
Facilities.	Facilities are to be well ventilated and free of fumes **(Must)**.	...	...	...
	Vessels and accessories contacting organisms and culture media made of non-toxic material **(Must)**.	...	...	...
	Culture facility located away from physical disturbances and preferably separated from test containers..	...	...	...
Apparatus.	Materials such as copper, brass, galvanized metal, lead, and natural rubber are not to come in contact with culture vessels or media, nor with test samples, test vessels, dilution water, or test solutions **(Must)**.	...	...	...
	All non-disposable test vessels and equipment to be thoroughly cleaned and rinsed in accordance with section 5.3 **(Must)**.	...	...	...
Stock Cultures.	Unialgal stock cultures of both males and females are maintained in separate, aerated 1000 mL Erlenmeyer flasks containing 800 mL of the culture medium (or same ratio of medium to vessel size) **(Must)**.	...	...	...
	Several cultures of both males and females are maintained simultaneously to keep a constant supply of plant material.	...	...	...
	To maintain vigourous growth, initial stock cultures are started periodically with about twenty 0.5 to 1.0 cm branch tips.	...	...	...
	Cultures are gently aerated through sterile, cotton-plugged, disposable, polystyrene 1 mL pipettes; and cultures are capped **(Must)**.	...	...	...
Media Renewal.	Media are changed at least once a week **(Must)**.	...	...	...
Culture Medium.	Culture medium is made up by dispensing filtered (0.45 µm) seawater into sterile flasks and adding the appropriate nutrients from sterile nutrient stock solutions (composition described at Table 1 of the USEPA document) **(Must)**.	...	...	...
Temperature.	23 ± 1 °C.	...	...	...
pH.	≥ 4.6.	...	...	...
Aeration.	Gentle, not exceeding 100 bubbles/min.	...	...	...
Light Quality.	Cool-white fluorescent.	...	...	...
Light Intensity.	75 µE/m^2/s.	...	...	...
Photoperiod.	16 ± 1h light; 8 ± 1h dark.	...	...	...
Salinity.	28 - 32 g/kg **(Must)**; ideally 30 g/kg.	...	...	...
QA/QC				
Validity Criteria.	Female control survival at the end of the recovery period is ≥ 80% **(Must)**. . .	...	...	...
	Mean number of cystocarps per control female plant is ≥ 10 **(Must)**.	...	...	...
Reference Toxicant. . .	Monthly and following the same procedure as the definitive test **(Must)**;	...	...	...
	ideally with organisms from culture that are used in toxicity test.	...	...	...
	Standard test with ICp endpoint **(Must)**.	...	...	...
	Sodium chloride, potassium chloride, cadmium chloride, copper sulfate, sodium dodecyl sulfate and potassium dichromate are suitable.	...	...	...
	Using same water as culture dilution/water.	...	...	...
Warning Chart.	Prepared for each reference toxicant and continually updated.	...	...	...
	ICp is acceptable if within warning limits (± 2 SD on log scale).	...	...	...
Sample Handling				
Volume.	Volume of 2L per test is required.	...	...	...
Containers.	Non-toxic materials for sample and transport containers, new containers or thoroughly rinsed used containers.	...	...	...
Labelling.	Upon collection, sample containers filled, sealed and labelled/coded.	...	...	...
	Include at least sample type, source, date and time of collection and name of sample collectors.	...	...	...

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May 1999

Parameter	Specification	Met Specifics? Y	N	NA
Holding Time.	Test to be initiated within 3 d after sampling **(Must)**; recommend within 1d. . .	...	...	...
Holding Conditions. . . .	Keep samples cool throughout their period of transport at 4 °C using regular ice or frozen gel packs.	...	...	...
	Upon collection, if sample > 4 °C, cool to 4 °C with regular ice or frozen gel packs (not dry ice).	...	...	...
	For renewal option only: the portion of sample required for solution renewal is to be stored in darkness in sealed container without air head space at 4 °C. .	...	...	...
Minimum Level of Reporting	**Do typical test reports reflect the minimum level of reporting outlined below? (Must)**.	...	...	...
Sample Data.	Brief description of sample type if and as provided to the lab.	...	...	...
	Information on labelling or coding of sample.	...	...	...
	Date of sample collection; date and time sample received at test facility.	...	...	...
	For effluent or leachate, T° of sample upon receipt at lab.	...	...	...
	D.O. and pH of sample just before its preparation and use.	...	...	...
Test Organisms.	Species and source of organisms.	...	...	...
	Any unusual appearance or treatment of test organisms, before their use in the test.	...	...	...
	Data showing health of organisms, including mean % mortality preceding test, presence of trichogynes in female plants, and presence of sori with spermatia in male plants.	...	...	...
Test Facilities.	Name and address of test laboratory.	...	...	...
	Name of person(s) performing the test.	...	...	...
	Brief description of test vessels (size, shape, type of material).	...	...	...
Control/Dilution Water.	Type and source of water used as control and dilution water.	...	...	...
	Type and quantity of any chemical(s) added to control or dilution water.	...	...	...
Test Method.	Statement that the Environment Canada guidance document on salinity adjustment has been followed.	...	...	...
	Citation of method used and type of test.	...	...	...
	In those instances where any sample or test solutions has/have been pH adjusted, and/or is/are filtered, brief description of procedure(s).	...	...	...
	Description of procedure(s) for salinity adjustment of sample and dilution water.	...	...	...
	Description of procedure for preparation of hypersaline brine.	...	...	...
	Design and description if specialized procedure (eg: renewal of solutions). . . .	...	...	...
	Frequency and type of all observations and measurements made during test.	...	...	...
	Name and citation of program(s) and methods used for calculating statistical endpoint.	...	...	...
Test Conditions.	Design and description if any deviation from or exclusion of any of the procedures and conditions specified in test method document.	...	...	...
	Number, concentration, volume, and depth of solutions in each test vessels. .	...	...	...
	# of individuals (male and female) per test vessel, and # of replicates per treatment.	...	...	...
	Statement (including procedure, rate, and duration) if any pre-aeration or aeration of sample or test solutions.	...	...	...
	Dates when test was started and ended.	...	...	...
Test Results.	All required measurements of T°, pH, D.O. and salinity in sample and test solutions (including HSB controls and, if natural seawater has been used as dilution water, natural seawater controls), before and made during the test. . .	...	...	...
	# and % of mortality of the female plants in each test chamber at the end of the recovery period.	...	...	...
	Mean number of cystocarps per female plant in each test chamber (± SD with corresponding coefficient of variation).	...	...	...

TEST SPECIFIC CHECKLIST

Test of Sexual Reproduction Using the Red Macroalga *Champia parvula*

Parameter	Specification	Met Specifics? Y	N	NA
Test Results (con't). . .	ICp (including the associated 95% confidence limits) determined for the cystocarp production data and indication of quantitative statistic method used; details regarding any transformation of data that was required.	...	...	...
	Results and duration of any toxicity tests with the reference toxicant(s) performed within 30 days of the test, together with the geometric mean value (± 2 SD) for the same reference toxicant(s) as derived at the test facility in previous tests.	...	...	...
	Anything unusual about the test, any problems encountered, any remedial measures taken.	...	...	...